Giving Plants Flounder Anti-Freeze

by Jeffrey H. Simonson

An edited version of this paper appeared in the Fairbanks Daily News-Miner Heartland section H-4, 23 Jan 94.

Plants like sunshine. Plants don't like freezing cold. Here in Alaska we have lots of both. The summers, though, are ideal for plant growth; they actually grow faster during Alaska summers than in more southern latitudes. This produces the Alaskan gigantic vegetables. Alaska's growing season is severely limited in both spring and autumn by freezing temperatures; extending the season by just a few days would allow plants to grow significantly bigger. Of course we can't change the length of our summer, but maybe we can change the plant's sensitivity to freezing.

Some animals have their own anti-freeze, enabling them to live in below-freezing conditions. Arctic fish survive by producing a protein keeping them alive down to -1.5°C. This anti-freeze protein, or AFP, is produced in their liver from a gene in their DNA. AFP works by binding to ice crystals and preventing further development. If we could copy this flounder gene, then insert it into a plant, then the plant might produce enough AFP to survive low temperatures. In 1990, Dr. Fawzy Georges and his staff at the National Research Council of Canada have done just that. They built a synthetic version of the AFP gene and inserted it into Black Mexican Sweet Corn in hopes of improving corn's cold hardiness. This procedure involves several complex steps: synthesizing the gene, construction of a suitable transfer vehicle, and introduction of the vehicle into corn plants.

To synthesize the gene, Dr. Georges used a new strategy. DNA is double stranded; normally, both strands must be synthesized. Dr. Georges synthesized just one strand; this method costs less than producing a double-stranded gene. To produce the AFP gene's complementary strand, he used the cell's own machinery. He simply used chemical enzymes taken from cells to synthetically replicate the gene in the same way done naturally.

The synthetic AFP gene was then joined with other segments of DNA to form a transfer vehicle, or plasmid. The plasmid also contained a promoter segment that told the corn cells to continually manufacture AFP. Interestingly, this promoter comes from a virus infecting cauliflower. Just after the AFP gene, Dr. Georges inserted a reporter gene called CAT. The CAT gene produces a protein that, when mixed with another chemical, turns blue. In this way, Dr. Georges could visually see when AFP was produced in the corn cells.

The plasmid containing the AFP gene was next introduced into corn. Separated plant cells, or protoplasts, however, have cell membranes not penetrable by DNA. So, Dr. Georges made holes in the protoplasts by a process called electroporation--the making of pores with electricity. He shocked the protoplasts twice with 250 volts. This killed many corn protoplasts, but in others, made holes just large enough for the plasmid to enter.

Determining if the corn protoplasts did indeed take up the plasmid and produced AFP is the CAT gene's job. Since the CAT gene immediately followed the AFP gene, then the presence of CAT protein indicates the presence of AFP. Dr. Georges broke open the corn protoplasts and mixed them with the CAT-indicating chemical. The chemical reaction showed presence of AFP.

Production of AFP within its cells is not, however, sufficient to give corn freeze tolerance. Cells must export AFP to the spaces between cells. The AFP protein was not exported because it apparently lacked the correct cell signaling sequence.

Though Dr. Georges's experiment with the flounder anti-freeze gene did not produce freeze-resistant corn, it did prove plants can produce anti-freeze proteins. This powerful technique is a first step to production of plants able to survive several days longer when cold temperatures return in autumn. In Alaska, this means plants may continue to grow when long daylight hours still persist, but temperatures have dropped to freezing. Alaska may someday produce significant agricultural crops using techniques of genetic engineering.